Steering soil microbiomes to suppress aboveground insect pests

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Abstract

Soil-borne microbes affect aboveground herbivorous insects through a cascade of molecular and chemical changes in the plant, but knowledge of these microbe-plant-insect interactions is mostly limited to one or a few microbial strains. Yet, the soil microbial community consists of thousands of unique taxa interacting in complex networks—the so-called microbiome—that provide plants with multiple beneficial functions. The role and management of whole microbiomes in plant-insect interactions are almost unexplored, calling for the integration of this complexity in aboveground-belowground research. Here, we propose holistic approaches to select soil microbiomes that can be used to protect plants from aboveground attackers.

Microbes conferring immunity in the phytobiome

The late entomologist, Thomas Eisner [1], once famously stated, "Bugs are not going to inherit the earth. They own it now". In light of on-going discoveries in microbial taxonomy and ecology, however, we can probably affirm that in fact "Microbes own the earth". The complex network of microorganisms inhabiting an area (e.g., soil, plant, animal), referred to as the **microbiome** (see Glossary), imparts crucial functions in all living organisms. For instance, the chemical defences that were previously considered an innate genetic feature of many animals and plants are actually produced by microbial symbionts [2, 3] and we expect more examples to be revealed in the near future. In humans, immunity, and even behaviour, are influenced by the intestinal microbiome [4, 5]. Interestingly, the **rhizosphere**, a thin interface between roots and soil, can be considered the plant equivalent to the human intestinal tract [6].

The soil is the major source of microbes, which determine the plant-associated microbiome [7]. Soil microbes are crucial for enhancing plant survival, growth, and tolerance to abiotic stress, but also induce systemic resistance (ISR) against pathogens and insects both aboveground [8-11] and belowground [12]. The soil microbiome has thus emerged as a key component of plant immunity [8, 9, 13], and shapes how plants interact with their abiotic and biotic environments, in the so-called **phytobiome** [14, 15]. Most of the work on aboveground plant defence, so far, focuses on the impact of individual microbial species or strains. This is in sharp contrast with DNA-sequencing techniques that are revealing an astonishing taxonomic diversity in soils, especially in the rhizosphere, but also the plant itself [7, 16, 17]. Because the beneficial effects for the plant are often provided by a consortium of microbes [18], there is an urgent need for approaches that incorporate the wider diversity that exists in nature into microbe-mediated plant protection strategies [19].

Impact of soil microbiomes on aboveground herbivores

Evidence for how belowground microbial communities, as a whole, impact aboveground insects is scarce; however, given the typically strong responses to only one or two experimentally augmented microbes, we anticipate that the community-wide effects are substantial. Soil microbiomes can impact aboveground insects indirectly through plant-mediated mechanisms, or directly through pathogenic or mutualistic interactions. A recent study showed that the population increase of the specialist foliar feeding aphid *Aphis jacobaea*, depended on the composition of microbial communities inhabiting the soil used by its host plant ragwort (*Senecio jacobaea*). The soils maintained different fungal communities that influenced the concentration of amino acids in the phloem sap, which the authors proposed, in turn, influenced the aphids [20]. Similarly, inoculation of distinct microbiomes collected from soils with different plant species altered the leaf metabolome of arabidopsis (*Arabidopsis thaliana)* and resistance of the plant to the caterpillar *Trichoplusia ni* [21]. This study further confirmed via removal of the majority of microorganisms using a filter of 0.45 µm, the contribution of the microbial component of the soil (instead of the presence of chemical compounds that could pass the filter) to plant performance. These studies illustrate that exposure to particular microbiomes alters the resistance of plants to aboveground insects (Figure 1, Key Figure). However, the underlying molecular plant mechanisms in microbiome-**induced systemic resistance** (ISR, Box 1) are probably more complex than predicted.

Soil microbes can have direct interactions with aboveground herbivores. Recent studies have shown that leaf and soil microbiomes are linked [22-24], and soils could thus influence the composition of insect pathogenic or symbiotic microbes present in or on the leaves. Entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae*, for example, are common in the soil but also exhibit an **endophytic** phase that can promote plant growth and insect resistance [25]. Remarkably, these fungi not only provide a benefit to plants by killing their herbivores, but can even translocate nitrogen from aboveground insect cadavers to the plant via fungal mycelia [26]. Other fungi historically considered to be limited to soils (e.g., *Trichoderma*) are now known to colonize leaves as endophytes where they can suppress insect pests such as thrips [27]. Insect symbionts provide their host with functions such as the ability to suppress plant defences or mobilize nutrients [28, 29], and these symbionts can be acquired via the soil. For example, the soybean insect pest *Riptortus pedestris* acquires *Burkholderia* strains from the soil that metabolize an organophosphate, conferring resistance to the insecticide [30].

Given the substantial evidence that soil communities affect aboveground plant interactions, we argue that agricultural scientists should start to think far more about reshaping microbiomes to increase crop resistance to insect pests. Managed systems allow a large amount of flexibility in inputs or other design strategies that shape soil life. Here, we focus on three specific strategies that are known to generate community-scale impacts on microbiomes and thus can be adapted for sustainable pest control aboveground.

Transplanting new microbiomes into the soil

A major advancement in microbe-plant interaction research was the development and commercialization of microbial inoculants for agricultural use. These inocula usually consist of one to several species that are phylogenetically clustered within a few genera (e.g., *Bacillus*, *Trichoderma*). However, many of these microbial inoculants that are successful under laboratory conditions fail when applied in the field. Recent studies have argued that this is probably due to competition of single strains with the existing microbiome in the donor soil [9, 31]. A potential solution to this problem would be to inoculate microbiomes that are more complex than currently used [19]. Large-scale cultivation of microbes and their introduction in complex synthetic microbiomes may aid in maximizing the beneficial functions of certain microbes by introducing taxa interactions [22, 32]. For instance, some microbes alter their metabolism when involved in microbial interactions, and produce compounds (e.g. volatiles, antibiotics) that are not produced when growing as single strains. These compounds could for example act antagonistically to other microbes that are prohibiting the establishment, enhance plant colonization, or have a direct effect on plant growth or resistance [33]. Soil microbial diversity is a major driver of ecosystem multifunctionality [34, 35] and due to the contribution of soil microbes to multiple functions such as nutrient cycling, biological control or food production, soil microbiomes are a multifunctional component of terrestrial ecosystems. Entire microbiomes can also be introduced via soil transplantation. A recent field experiment showed that introducing a thin layer of soil (5 mm) resulted in accelerated nature restoration in a degraded ecosystem, and that composition of the bacterial and fungal communities six years after application was still different from those were no soil was added [36]. Other studies show that soils with disease-suppressive properties can be successfully transplanted and remain disease suppressive in the new area [9, 17]. Agricultural soils, in particular in commercial glasshouses, are regularly sterilized, e.g., by steaming. This practice eradicates much of the existing microbial community [37], a situation that is ideal for introduction of a new microbiome.

There is a unique opportunity here to forge collaborative and mutually beneficial relationships among those studying plant and animal microbiomes. Faecal microbiota transplantation is now frequently used to suppress diseases and alter immune responses in humans while soil inoculation and transplantation is still in its infancy. Hence, those studying human health consequences of gut microbiome transplantation are far ahead of those working in plant health. Yet, the two approaches, while differing in practical aspects of implementation, are identical in theory. In fact, direct analogies between these two areas have been highlighted for characteristics such as nutrient uptake, pathogen defence, and immune function [6, 38].

Steering existing soil microbiomes

Apart from introducing a new microbiome, the residing soil community can also be steered to a desired beneficial state [39, 40]. This could be accomplished by stimulating particular subgroups of the microbiome via manipulations of environmental factors such as soil temperature or moisture levels [37], via the application of chemical compounds or manipulating resource availability through organic amendments. It is well known that amelioration of soils with manure or plant residues alters the soil microbiome, thereby suppressing belowground pathogens [9, 40, 41]. Different studies have shown that addition of biochar, pyrolized plant residues, to soil, for example, increases bacterial diversity and microbial biomass [42], as well as resistance of plants against aboveground pests and diseases [43, 44].

Interestingly, the plant response that biochar causes to the pathogen *Botrytis cinerea* highly resembles microbial-ISR, including **priming** of defence-related genes associated with the early oxidative burst via the jasmonic acid (JA) signalling pathway [43]. These set of studies highlight how a soil amendment could impact aboveground attackers through changes in the soil microbiome and in plant defences. However, evidence linking how soil amendments alter the soil microbiome, and how this cascades to induce systemic resistance in plants is still missing.

Certain “keystone” microbes are highly connected with other taxa and play a key ecological role in the microbiome. By targeting keystone species the entire microbial network can be adapted and recent discoveries support this idea [32, 45, 46]. Introduction of the oomycete pathogen *Albugo* sp. and the basidiomycete yeast fungus *Dioszegia* sp., for example, alters the microbiome network in the **phyllosphere** of arabidopsis [45]. The important role of these keystone taxa suggests that they should be present in high abundance in the microbiome. However, keystone species can also play an important role at low densities and even rare microbes, which have been shown to induce resistance against aphids [47], can act as keystone players in microbiomes [48]. Whether a microbial function such as induced systemic resistance after introducing a keystone taxa is driven by changes in the microbiome network, rather than by the introduced taxa itself, is still unknown.

Using plants to steer the soil microbiome

By growing in the soil, plants modify the microbiome, either directly, or indirectly via influencing the abiotic environment [7]. Host factors such as plant species, ontogeny, and exposure to antagonists all shape microbiomes. Even different genotypes imprint unique microbial signatures on the soil [7, 24, 49-51]. Plant roots release compounds such as sugars, organic acids, phytohormones, and secondary metabolites, and this **exudation** influences the soil community [52, 53]. For instance, specific compounds (e.g., malic acid, benzoxiacinoids, strigolactones) can enhance or recruit certain beneficial soil microbes in the rhizosphere [54-57]. Interestingly, the exudation of some of these compounds increases following aboveground herbivory, suggesting this is an active strategy whereby plants recruit beneficial microbes for protection. The impact of herbivory on the soil can also influence the susceptibility of plants that are later exposed to this microbiome [58, 59]. For example, the soil fungal community in the rhizosphere of ragwort that suffered from belowground or aboveground herbivory differed considerably from communities in unexposed plants. Plants that grew later in the soil with a belowground herbivory legacy displayed higher resistance to the leaf chewer *Mamestra brassicae*, and this was associated with a modified profile of pyrrolizidine alkaloids in the foliage [59]. These two examples illustrate a closed feedback loop in interactions between plants, soil microbes, and insects, a term that we propose to call “**plant-soil-insect feedbacks**”.

The concept of plants changing the soil microbiome, which subsequently influences the performance of other plants that grow later in the soil is one of the main mechanisms of “**plant-soil feedback**” [60, 61] and is the basis for ancient agricultural practices such as crop rotation, intercropping or cover crops. However, this concept has primarily been used in the context of avoidance of soil pathogen build-up and autotoxicity, or to increase nutrient availability by using leguminous crops. We argue that plants displaying positive feedback effects on crop immunity to pests through their effect on the soil microbiome, should be selected for and included in rotation systems, as “engineers” of beneficial soil microbiomes. These plants that create a beneficial microbiome with positive effects on plant health can also be used to produce inocula that can be then be introduced during or at the start of cultivation. Surprisingly, the contribution of soil microbiomes to plant-soil feedbacks and their application in agriculture is largely unknown [62]. There is an urgent need for studies that improve our understanding of the mechanisms by which plants influence soil microbiomes and that predict how plants respond to these changes (see Box 2). This will enable us to design optimal combinations of succeeding plants in rotation schemes and enable breeding for optimal crop responses to soil manipulations [63].

The genetic traits that underlie the responses of plants to changes in soil microbiomes are also largely unknown. However, a recent genome-wide association study in arabidopsis identified ten genetic loci that were highly associated with the ability of the plant to respond to the growth-promotion effect of volatiles from a soil derived *Pseudomonas simiae* strain [64]. In crop plants, breeding for resistance to pathogens in combination with high inputs of fertilizers and pesticides that suppress pathogens and herbivores, may have selected for poorly responding genotypes, and even for genotypes that suppress beneficial microbes [49, 65]. Therefore incorporating knowledge about microbiomes during the crop selection process may improve traits such as plant productivity and resistance. By growing plants repeatedly in the soil and selecting in each generation for specific plant traits such as early onset of flowering or more efficient induction of defences, beneficial soil microbiomes can be selected and therefore further steered, so that they become more effective [17, 66, 67].

Concluding remarks and future perspectives

Unravelling the mechanisms that govern species interactions is a major challenge in ecology. In this opinion we have emphasized that soil microbiomes can be manipulated to enhance plant performance and resistance to aboveground pests, and that plants play pivotal roles in this. The mechanisms can be diverse, as soil microbiomes are complex entities, and include priming for enhanced defensive responses, induction of plant secondary metabolites, as well as direct interactions between soil microbes and insects (via direct contact of insects with the soil or via colonization of plant by soil microbes). We propose three areas for future research that are essential if we aim to steer microbiomes to alter aboveground plant-insect interactions (see also Outstanding Questions).

First, fundamental knowledge on the mechanisms of how plants shape soil and plant microbiomes will help to develop new approaches and products. For instance, cultivars emitting higher levels of compounds that enrich certain groups of beneficial microbes could be selected, or products based on those of compounds could be developed. Also, breeding programs could select plants to enhance microbe-mediated functions, from leaving positive soil legacies to strongly respond to these legacies by increasing growth or inducing resistance in aboveground tissues [63]. Therefore, knowledge about soil, plant and insect microbiomes should be integrated into established research on insect-plant interactions to fully understand the functioning of these interactions within the phytobiome.

Second, in a similar way as gut microbiome transplantation in humans has been a major breakthrough in overcoming recurrent *Clostridium difficile* infection [68], we propose that soil microbiome transplantation can be successful to induce resistance in plants against insects. Plant-soil feedback concepts can be used to create specific donor soils. We envisage that in agriculture, plants will be grown with a clear purpose of conditioning soil that can be transplanted, or that soil conditioning will be incorporated in crop rotation systems. A major challenge is to predict which plant species or genotypes can be used to obtain desired soils. Understanding microbiome assembly and function in different plants, coupled with empirical knowledge on agricultural practices, and on microbe-plant-insect interactions, will be essential for the development of such predictive models.

Third, we propose that since insect herbivores can severely impact productivity in terrestrial ecosystems, plant resistance to insects should be seen as a key service of microbiomes, and microbiome-insect interactions should be included in agricultural management strategies. Many of the ecosystem services of soil microbiomes may not be effective under current production systems with high input of pesticides and chemical fertilizers, and only become apparent when plants are exposed to abiotic stress conditions [19]. Based on current global changes in agriculture and nutrient supplies, we expect that beneficial soil microbiomes will play an even more important role in plant productivity in the future. The increased availability of nutrients in agriculture has been the basis for the first green revolution that led to a boost in yields worldwide. We are now at the verge of a second green revolution, which utilizes the potential of microbiomes to boost plant health and productivity [69, 70]. The service of plant and soil microbiomes to induce resistance in plants to insect pests should be an essential part of this second green revolution.

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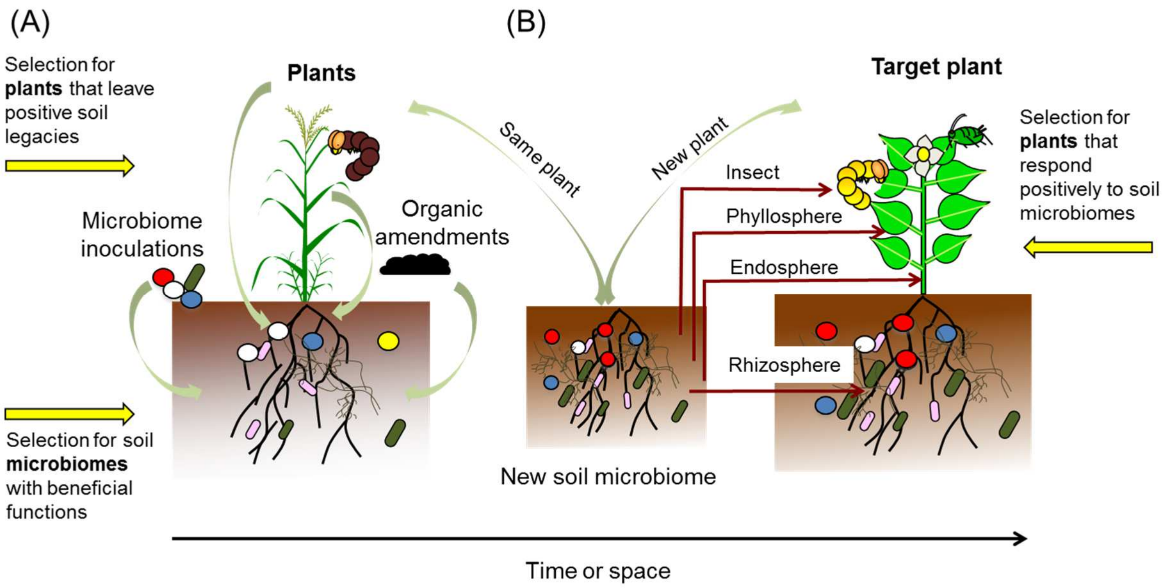
Figure 1



(A)
(B)
Selection for **plants** that leave positive soil legacies
Plants
Microbiome inoculations
Organic amendments
Selection for soil **microbiomes** with beneficial functions
Same plant
New plant
Target plant
Insect
Phyllosphere
Endosphere
Rhizosphere
Selection for **plants** that respond positively to soil microbiomes
New soil microbiome
Time or space

Figure legend

Figure 1. Soil microbiome manipulation to induce resistance in plants against aboveground insects. (A) Soil microbiomes can be steered by different strategies such as inoculating new microbiomes, adding organic amendments, or by growing certain plants. Interactions of the plant with antagonists such as aboveground insect herbivores will further shape the soil microbial community. Different components of the system can be selected for desirable traits. For instance, through plant breeding, cultivars that recruit beneficial soil microbiomes can be developed. Soil microbiomes can also be engineered, selecting through several generations those soils that confer plants with certain functions. (B) The new microbiome can affect plant growth and resistance to aboveground attackers of the plant that is already growing in the soil, but also that of plants growing later in the soil. The new soil microbiome will be an important source for the microbial assembly of the rhizosphere, endosphere, and phyllosphere of plants. Microbes inhabiting those habitats can suppress aboveground insect pests, either directly (e.g. insect pathogens) or indirectly via changes in the immunity of the host plant. Cultivars that show strong positive responses (in terms of plant growth, resistance, etc.) to soil microbiomes could be developed. The suggested pattern of events could happen along a temporal (e.g. in a crop rotation system) or spatial axis (e.g. during intercropping).

Glossary:

Endophytic: that colonizes inside above- and/or belowground plant organs, without causing evident disease symptoms.

Endosphere: microbial habitat inside plant organs.

Induced systemic resistance (ISR): enhanced resistance in the entire plant against pathogens and herbivores, characterized by priming, and triggered by beneficial microbes.

Microbiome: totality of microbial genomes present in a particular environment, for example soil, rhizosphere, phyllosphere or endophytic compartment.

Phyllosphere: the surface of aerial plant organs, dominated by the leaves.

Phytobiomes: plants, their environment, and their associated communities of organisms, including microbes, animals, and other plants.

Plant-soil feedbacks: changes by a plant in the biotic and abiotic characteristics of the soil they grow in that influence the next generation of plants growing in the same soil.

Plant-soil-insect feedbacks: plant-soil feedbacks that have effects on insects, or that are affected by insect feeding on the plant creating the soil legacy.

Priming: alert state after certain stimulus that allows plants to mount a stronger and/or faster defensive response upon attack.

Rhizosphere: thin layer of soil in contact with roots, that is under direct influence of root exudates and soil microbes.

Root exudates: molecules released by plant roots and that among others, include organic acids and sugars.

Box 1. Microbial-induced systemic resistance against insects

Plants can induce several types of resistance upon interacting with herbivores, pathogens, or beneficial microbes. From those, induced systemic resistance (ISR) is the enhanced defensive capacity of the entire plant against a broad spectrum of attackers triggered upon local induction by beneficial microbes [69]. Plants then enter in a primed state that allows them to respond faster and stronger upon herbivore or pathogen attack [11]. Our knowledge on the molecular mechanisms of ISR against insects has substantially increased in recent years. Several microbes, including plant-growth promoting rhizobacteria, mycorrhizal fungi, and free-living fungi such as *Trichoderma,* can trigger ISR against insect herbivores and especially against generalist leaf chewers. Interestingly, the mechanisms seem to be conserved across microbial groups. However, although in most cases ISR against insects is regulated by JA- and ET-signalling pathways [69, 71], some microbial strains require other signalling pathways to be functional [72]. Genes such as *LOX2*, *PDF1.2*, and *HEL*, are often more strongly induced after herbivory in arabidopsis plants that are inoculated with plant growth-promoting rhizobacteria [71, 73, 74]. However, the effects and underlying mechanisms of microbes on insects are highly diverse, and two aspects in particular suggest that the established paradigm of ISR needs to be re-evaluated: (i) *Direct induction instead of priming*: Soil microbes can also directly induce plant defence responses in the absence of an attacker. Genes in the ET-pathway such as *ORA59* and *PDF1.2*, for example, are induced by rhizobacteria colonization in arabidopsis [71], or the JA-regulated genes *GhAOS*, *GhLOX1* and *GhOPR3* in cotton [75]. Associated with this, plant growth-promoting rhizobacteria or their volatiles directly induced the synthesis of glucosinolates in arabidopsis [71, 72, 76] and gossypol in cotton [75]. (ii) *Induced systemic susceptibility*: insect performance often increases upon soil inoculation with beneficial microbes. This is especially common in phloem feeders such as aphids and whiteflies, probably due to their behaviour that avoids damaging cells and feeding on phloem sap with lower levels of defensive compounds than the overall leaf tissue [10]. But microbe-induced susceptibility has also been observed in generalist caterpillars [77, 78]. Elucidating the factors causing this variability will be a major breakthrough in the knowledge and application of microbe-plant-insect interactions. Similar to microbial interaction networks, insects and plants are also structured in interaction networks. Systems approaches coupling microbial, insect and plant signalling networks will allow scientists to design predictive models of microbiome-plant-insect interactions.

Box 2. Plant-soil and plant-soil-insect feedbacks

Plants as primary producers provide the basic resources for soil biota, including insects, nematodes and microbes [79]. They contribute litter originating from dead shoots or roots to the soil, and living plant roots release an array of metabolites. Via these effects, plants shape soil biotic communities that use these compounds or are influenced by them, and alter the physical and chemical properties of soils. These plant-mediated changes of the soil can influence the performance of other plants that grow later in the soil [60, 61]. This phenomenon is called plant-soil feedback and is now receiving considerable attention because of its relevance in vegetation dynamics and invasion ecology. Plants can affect individuals of the same species (known as direct or conspecific feedback) or of different species (indirect or heterospecific feedback). Most examples of conspecific plant-soil feedbacks are negative, but heterospecific soil feedbacks are often positive, since many species perform better in soil conditioned by others than by its own species [60, 61]. Outcomes also vary widely between plant species and soils, and more research is needed to predict these patterns. Plant functional traits such as growth rate, specific root length, and even aboveground characteristics such specific leaf area, have been used to predict plant soil feedbacks in natural ecosystems. For instance, soil conditioned by fast-growing plant species or those with higher belowground biomass produced more positive feedbacks due to increased nitrogen availability [80, 81]. One of the most straightforward predictions is that closely related plant species have a higher chance to be attacked by similar pathogenic microbes, and negative feedbacks would be expected in this case. However, studies so far show inconsistent effects of the relationship between phylogenetic relatedness and plant-soil feedbacks [81-83]. Another layer of complexity in plant-soil feedbacks are the presence of herbivorous insects attacking the plants involved in the feedback, a concept that we would like to define as plant-soil-insect feedbacks. A first possibility is that herbivory on the plants that condition the soil alters soil legacies [59]. The second possibility is that plant-soil feedback effects cascade to insects interacting with the responding plant during the feedback phase [20]. Both scenarios may occur in a single plant-insect system [59]. Ecological knowledge of plant-soil feedback effects on natural enemies of plants has strong potential for future implementation in agricultural ecosystems.

Outstanding Questions Box.

- Can we develop a universal approach to manage soil and plant microbiomes to achieve higher yield, tolerance to abiotic stress and enhanced resistance to pests?
- What genetic, molecular, and chemical plant mechanisms are responsible for how plants shape and respond to soil microbiomes?
- What are the mechanisms that underlie microbiome-induced systemic resistance to aboveground attackers and what are the consequences for higher trophic levels?
- How do soil microbiomes interact with plant- and herbivore-associated microbiomes to influence plant-insect interactions?

Trends box.

- Soil microbes are a major source of the plant microbiome and recent advances show that they are key component of plant resistance against aboveground attackers
- However, most of our knowledge on how belowground microbes affect aboveground pests is limited to single strain effects, calling for research that incorporates the full potential of the entire soil microbiome.
- Soil microbiomes can be manipulated, as done for centuries through agricultural practices as crop rotation or the use of amendments. Conditioned soils can be transplanted to restore ecological functions in other ecosystems.
- The role of the plant in shaping soil microbiomes and in how they respond to them can be maximized but we need to increase our mechanistic understanding at genetic, physiological and ecological levels.